Current status of transgenic animal research for human health applications

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ABSTRACT

Background: The current status of genetic engineering animals for biomedical and human health applications, including improving the supply of animal-based food products, was reviewed. Although transgenic animals have been available for almost 30 years only one product, a drug derived from transgenic goats’ milk, has been approved for use anywhere in the world. While a number of technical issues limited efficiency initially, products coming to market were hindered by the lack of a regulatory framework and compounded by opposition from anti-biotechnology groups.

Review: As presented in the review, the production of genetically engineered livestock has progressed from the initial technology of pronuclear microinjection through to the wide-spread use today of somatic cell nuclear transfer-based cloning technology following the transfection of cells in culture with methods such as lipofection or electroporation. There also was significant progress in the development of systems based upon lentivirus or adeno-associated virus-based vectors. More recently, advances occurred based on transposon-mediated transgenesis. In conjunction with advances in methods to genetically engineer livestock, there are also advances, such as the use of zinc-finger nucleases and transient depletion of endogenous non-homologous recombination systems, to increase the efficiency of homologous recombination-based gene targeting. Applications range from the production of pharmaceutical proteins, silk for use in sutures and scaffolds for cellular regeneration, and xenotransplantation through to the development of transgenic animals that improve animal production and the nutritional value of animal-based food products. Both of these agricultural applications are important for maintaining the global production of sufficient meat-based products in an economically and environmentally sustainable manner in the face of a dramatically increasing human population.

Conclusion: Advances in the production of genetically engineered animals and identification of useful applications have significantly improved and have set the stage for the adoption of transgenic animals. Brazil, because of recent technological developments and the adoption of regulatory guidelines is now in a strong position to benefit from genetically engineered animals and to contribute to the leadership in the adoption of transgenic animals for applications to improve human and animal health and well-being.

Keywords: genetic engineering, transgenic, livestock, applications.

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I. INTRODUCTION

Since the initial production of the first genetically engineered (GE) or transgenic livestock in the mid to late 1980s [12,26,31], there has been the promise that this new technology would provide substantial benefits to human health. These benefits would be derived both through the development and production of biomedical products and through the development of improved animals for use in agriculture. Food animals may be genetically engineered to result in animal-based food that improves nutrition or delivers nutriceuticals to the consumer. However, to date only one biomedical product, the drug ATryn® [7], and no transgenic animals have been approved for use in agriculture anywhere in the world, in part because of misunderstandings concerning the technology and opposition by some anti-biotechnology groups [27]. Here, we will discuss current methodologies and applications of GE livestock that are waiting to fulfill the promise of this technology.

II. PRODUCTION OF GE LIVESTOCK

The first genetically modified animals were produced using pronuclear microinjection; however, this technique is very inefficient with only 1-5% of live-born animals being transgenic across the various livestock species [5] and results in the random integration of the transgene in the genome, thus only allowing for gene addition. The advent of somatic cell nuclear transfer (SCNT) technology has advanced the production of transgenic animals and is the most viable option for producing GE livestock with gene-specific modifications [3]. In SCNT, cells are genetically modified prior to nuclear transfer into an enucleated oocyte and implantation in a surrogate host. In the past, electroporation and chemical transfection methods such as lipofection were used to deliver transgene constructs into the cells [20]. Recently, more efficient methods of transgene delivery have been developed, such as transposon-mediated transgenesis [15] and viral-mediated delivery methods using lentiviral vectors [37] or the newer recombinant adeno-associated virus (rAAV) [30]. To date, multiple livestock species carrying a variety of transgenes have successfully been produced using these methods [8,19].

Non-targeted integration, while successful for producing a variety of transgenic animals, has associated issues such as integration at transcriptionally silent genomic sites and the risk of disrupting important genes in the host, which may decrease the efficiency of producing live, expressing animals. Efforts have gone into improving targeted transgene integration, which allows for more precise genomic manipulation. Strategies employed to increase gene targeting efficiency include promoter or gene traps [25,32], positive-negative selection [13,24], transient manipulation of DNA recombination pathways [2,34], site-specific recombinase systems [10], and zinc finger nucleases [16,39] – the last of which shows impressive gene targeting efficiencies. In conclusion, we now can reliably produce transgenic animals in all the main species used in agriculture. We have the capability to insert genes and produce targeted insertions; however, targeted transgene integration is still inefficient. Therefore, while recent advances have been encouraging, more research is necessary to improve gene targeting efficiency.
III. BIOMEDICAL APPLICATIONS

Genetically engineered livestock (cows, goats, sheep, pigs or chickens) present a unique platform for producing a wide variety of medically-relevant products. The most popular system uses the mammary gland of a transgenic animal as a protein bioreactor, however, there are alternative systems based on producing useful pharmaceutical products in blood and eggs as well. The main system currently used to produce relevant human therapeutic proteins is mammalian cells in culture. Mammalian cells are difficult and expensive to grow in liquid media, but they are used because most therapeutic proteins must go through post-translational modifications in order to be functional, a process that cannot be performed by bacterial cells. Cell culture-based bioreactors also require significant overhead and constant attention and monitoring. Transgenic animals are an attractive alternative because animals are naturally self-regulating through maintaining homeostasis. Producing recombinant proteins in milk, blood, or eggs also can result in higher levels of the recombinant protein that can be easily harvested and thus animals can be used as more cost-efficient bioreactors for therapeutic proteins than typical cell culture-based bioreactors.

As mentioned earlier, currently there is one therapeutic protein on the market that is isolated from the milk of transgenic goats; there are many others that are being tested, but for the purpose of this review we will only touch on a few. The first drug produced by a transgenic animal to achieve market approval is ATryn® produced by GTC Biotherapeutics. ATryn® is a recombinant human antithrombin produced in transgenic goats’ milk that treats deep vein thrombosis [7]. The bio-defense company PharmAthene is producing Protexia®, a recombinant human butyrylcholinesterase (rBChE) also in the milk of transgenic goats [14]. Butyrylcholinesterase is a potent organophosphorous scavenger protein that is being developed for use as a prophylactic against acute organophosphorous nerve agent toxicity. It is naturally produced in humans, however in very low levels that would not be sufficient to protect the body against damage in the event of an exposure to nerve gas. Supplementing with rBChE has been shown to prevent damage caused by nerve gas.

Transgenic goats are also being used to produce growth factors such as human granulocyte colony stimulating factor (hG-CSF), which stimulates the production of white blood cells and hematopoietic stem cells. These transgenic goats were created at the State University of Ceará and were the first transgenic goats created in Latin America [9]. Along with producing novel therapeutic proteins, transgenic goats are also being used to produce spider silk in their milk, which can be filtered out and used for bio-matrices and sutures [38]. These goats are currently at the University of Wyoming, where filtration processes to remove the silk protein from the milk are being investigated.

In addition to using the mammary gland as a bioreactor to produce pharmaceutical proteins, transgenic animals are also being used to produce human antibodies. Hematech is a company that is using transgenic cows to create human polyclonal antibodies in the cows’ blood, which can then be harvested [17]. These polyclonal antibodies can be used for a variety of different purposes, including treatment for bacterial infections or providing immune protection to immuno-deficient patients. In another approach to produce polyclonal antibodies, the company ImmunoGenes is generating transgenic rabbits and sheep over expressing the neonatal Fc receptor (FcRn). The Fc receptor binds to IgGs and albumin, protecting them from degradation by capillary endothelial cells. Over expression of this receptor increases the number of protective Fc receptors that are available to bind the antibodies, which can increase the antibody yield significantly [1].

Finally, chicken eggs also can be used as a way to package and harvest novel proteins from transgenic chickens. The Roslin Institute in Edinburgh leads in this technology, using a lentiviral vector system to genetically engineer chickens [21]. This system has allowed for the creation of chickens that express therapeutic proteins only in the gland cells of the oviduct, which are the cells that produce over fifty percent of the protein that goes into egg whites.

IV. XENOTRANSPLANTATION

The other category of medically relevant products made by transgenic animals is generating animal tissue and organs for use in human transplants, or xenotransplantation. There is a severe shortage of healthy replacement...
organisms and tissues and as the baby boomer generation ages the demand for replacement organs will rise. Transgenic animals offer a reliable and economic way to meet this growing need. The largest obstacle facing this technology is overcoming hyper-acute rejection and acute vascular rejection. These immune responses can cause rejection of an organ in a matter of minutes to within a few weeks, respectively. Revivicor, a company that specializes in regenerative medicine, has created galactosyl transferase double knockout pigs [28]. This knockout eliminates the Galα1,3 sugars that are found on the surface of the pig’s organs that causes hyper-acute rejection. Without these sugars the human body does not identify the organ as foreign, thus solving the problem of hyper-acute rejection. To address the problem of acute vascular rejection, multi-gene transgenic pigs have been produced that regulate humoral responses to non-galactosyl-based antigens, inhibit coagulation and thrombosis, and provide local protection against human cellular response [33]. These transgenes, if expressed constitutively in the body could have adverse effects in the pigs and therefore tissue specific promoters were used to allow expression in certain applicable tissue types, such as the heart and pancreatic islets. These animals could provide a wide variety of organs and tissue including hearts, kidneys, surgical mesh, and orthopedic tissue.

There are many potential uses, and therefore opportunities, for transgenic animals in the field of medicine. As the technology advances and more products produced by transgenic animals are approved, we expect to see a rise in the use of transgenic animals for biomedical applications.

V. PRODUCTION AGRICULTURE

Having a nutritious, plentiful, and healthy food supply is another component of ensuring the health of humans. A major challenge globally will be to produce sufficient food in an environmentally and economically sustainable manner to feed the world’s burgeoning human population in the next 25 to 50 years. Genetic engineering of animals will certainly play a role in the development of suitable livestock and aquaculture strains to help meet this need. The current and potential applications of transgenic animals in agriculture including increasing the efficiency of production, improving animal health and welfare, and altering the nutritional value of animal-based products, have been recently reviewed [8,19,35], so remarks here will be restricted to only a few examples.

In terms of providing a healthy, sustainable supply of animal-based food products, GH-transgenic Atlantic salmon are now in an advanced stage of regulatory review by the FDA and may become the first transgenic animal product approved for human consumption in the U.S. The incorporation of a Chinook salmon GH construct in this line produce salmon that reach market weight faster and are more feed efficient than wild-type salmon [6] and may become an important source of fish product for the world’s market in the face of declining wild stocks. Lines of GE pigs also have been developed to improve the efficiency and sustainability of pork production [36] and to reduce the environmental footprint associated with swine production [11] and, again, these types of GE animals may play an important part in maintaining the supply of animal products for human consumption in the coming decades.

Another promising application of transgenic animals is the potential for the development of animal-based food products that contain functional components that enhance food safety or nutritional value, and thereby improve human health. For example, the potential to decrease the amount of deleterious fats and cholesterol in milk [29] or increase the prevalence beneficial fatty acids in pork tissue [18] has been demonstrated and is consistent with dietary recommendations for cardiovascular health. While neither of these projects has progressed to the point of having a useful line of transgenic animal for use in agriculture, the preliminary work clearly suggests that alterations may be possible that could have significant human health implications.

Finally, genetic engineering can be used to introduce beneficial proteins into tissues, milk or eggs used in animal-based foods. For example, the expression of human lysozyme in transgenic goats’ milk [23] has several potential benefits, including improving udder health, increased shelf-life of the milk, and increased gastrointestinal health of young animals or humans consuming the milk. Milk from the transgenic goats contains approximately 68% of the lysozyme found in normal human milk and has significant antimicrobial properties, both in vitro [22] and when consumed as pasteurized fluid milk [4]. The data from animal models indicate that all the potential benefits are likely to be achieved, including the potential gastrointestinal benefits for humans. According to the WHO, over two million children die yearly from diarrheal diseases, with E. coli being one of the major causative organisms. Some strains of this is a bacterium, whose growth and viability is clearly inhibited in vivo by the consumption of pasteurized human lysozyme-containing goats’ milk when consumed by young pigs as a model for human health, is a major cause of debilitating diarrhea.
VI. CONCLUSIONS

Developments for the identification of gene function and promoter systems are progressing rapidly due to the worldwide focus on mammalian genomics. This fact, when coupled with the advances such as SCNT-based cloning, lentiviral vectors, and zinc-finger nucleases, foreshadow a resurgence in interest in the application of GE in agricultural animals. Brazil is well placed to play a significant role in this field as it has the technological base and laboratories actively involved in cloning livestock and producing transgenic animals. Finally, and perhaps most importantly, the Brazilian federal government has moved forward with guidelines and rules for the assessment and use of GE animals for biomedical and agricultural applications.

REFERENCES


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